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Interactions of Guanine Derivatives with Ethylenediamine and Diethylenetriamine Complexes of Palladium(II) in Solution: Pd Binding Sites of the Guanine Ring and Formation of a Cyclic Adduct, [{Pd(en)(guanine ring)}$_4$]

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Interactions of guanine derivatives (G) with ethylenediamine (en) and diethylenetriamine (dien) complexes of Pd(II) in aqueous solution were investigated by Raman, IR, CD, and NMR (^{15}N, ^{1}H, ^{31}P) spectroscopy and by chromatography. The Raman and IR spectra varied with the molar ratio r of Pd to G, and two types (A and B) of coordination of G to Pd were found. Detailed analysis of the Raman and IR spectra including those of ^{18}O=C(6)-substituted guanosine showed that N(7) is the binding site in adduct A and both N(7) and deprotonated N(1) are bound to different Pd atoms in the adduct of Pd(dien) at r = 2 (B1) and the adduct of Pd(en) at r = 1 (B2). ^{15}N NMR spectra supported the binding at N(7) and deprotonated N(1) in the B1 and B2 adducts. Gel chromatography and HPLC suggested that B1 consists of two Pd(dien) molecules and one guanine ring, while B2 is an n/n adduct of Pd(en) and G. ^{1}H NMR spectra of B2 and its analogues formed with mixtures of guanosine 5′-monophosphate and inosine 5′-monophosphate can be interpreted as being due to a 4/4 cyclic adduct with C_4 symmetry in the G ring configuration. Reflecting the fixed configuration of the G rings, strong CD bands are observed for this adduct. ^{31}P NMR and vibrational spectra suggested that the phosphate group of guanosine 5′-monophosphate is hydrogen bonded from the amino group of Pd(en), and this hydrogen bonding stabilizes the 4/4 cyclic adduct.

Introduction

A platinum complex, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), is an anticancer drug, and its principal target has been considered to be DNA, especially guanine bases.[1,2] For elucidation of the action mechanism of the drug, interactions of the Pt(II) complex and its analogues with DNA or guanine derivatives have been studied extensively.[2-19] However, the way of binding of the complex to guanine derivatives (G) in solution has not been fully clarified. Because of the low solubility and reactivity in water, it takes several days for the Pt(II) complexes to form thermodynamically stable adducts with guanine derivatives. Furthermore, coexistence of various adducts in the intermediate reaction stages complicates the detailed structural analysis of the adducts.[8-10] As more soluble and reactive substitutes,[20] Pd(II) complexes are useful in the studies of metal–nucleotide interactions. The binding modes of Pd(II) complexes may serve as references for those of slowly reacting Pt complexes.

For the modes of Pd(II) binding to the guanine ring, several models have been proposed mainly by using IR and ^{1}H NMR data. The models include chelation, where one Pd ion binds to N(7) and C(6)O of a guanine ring,[21,22] polymerization with C(6)O–Pd–N(7)′ bridges between adjacent guanine rings,[23] and mononuclear or binuclear adduct formation with Pd–N(7), Pd–N(1), N(7)–Pd–N(7)′, or N(1)–Pd–N(1)′ linkages.[24-26] In order to specify the binding modes, studies by other physicochemical methods are required.

We have investigated the interactions of Pd(II) with guanine derivatives, guanosine 5′-monophosphate (GMP) and guanosine (Guo), in aqueous solution by Raman, IR, CD, and NMR (^{15}N, ^{1}H, ^{31}P) spectroscopy and by chromatography. The Pd(II) complexes used are [Pd(dien)(NO_3)]NO_3 and [Pd(en)(NO_3)$_2$], where dien and en stand for diethylenetriamine and ethylenediamine, respectively (see Figure 1). The NO_3 ligands of these complexes are labile, and Pd(dien) provides one binding site for G, while Pd(en) provides, at most, two sites in the cis configuration. On the basis of the Raman and IR spectral patterns, the Pd–G adducts have been classified into two types, A and B. The B-type adduct is further classified into two subgroups B1 and B2. In the 1/1 adduct of Pd(dien) and G (adduct A), N(7) of the guanine ring is found to be the binding site. Vibrational and ^{15}N NMR spectra have given evidence that the guanine rings of both the B1 and B2 adducts coordinate to Pd at N(7) and deprotonated N(1). CD, NMR (^{1}H, ^{31}P), and chromatography studies on the structural differences between B1 and B2 have led to the conclusion that the structure of B1 is Pd(dien)–G–Pd(dien) and B2 has a 4/4 cyclic structure of [{Pd(en)(G)}$_4$].

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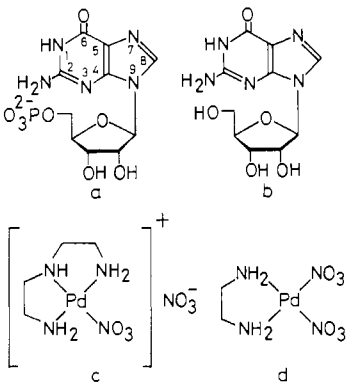

Figure 1. Schematic structures of (a) guanosine 5′-monophosphate (GMP), (b) guanosine (Guo), (c) (diethylenetriamine)nitratopalladium(II) nitrate, $[Pd(dien)(NO_3)]NO_3$, and (d) (ethylenediamine)dinitratopalladium(II), $[Pd(en)(NO_3)_2]$.

Experimental Section

Materials. The Pd(II) complexes, $[Pd(dien)(NO_3)]NO_3$, $[Pd(en)(NO_3)_2]$, and $[Pd(tmen)(NO_3)_2]$, were synthesized according to the literature (tmen = *N,N,N′,N′*-tetramethylethylenediamine).[27-29] Guo, GMP, inosine 5′-monophosphate (IMP), and inosine (Ino) were obtained from Kohjin Co. and were recrystallized from water except for Ino. Ino was recrystallized from ethanol/water (8/2). The synthesis of ^{18}O=C-(6)-substituted guanosine (^{18}O-Guo) followed the literature method.[30] Deuteriation at C(8) of the guanine ring was performed by heating the guanine derivatives in D_2O at 80 °C for 24 h.[31]

Sample Preparation. Samples of a Pd complex and a nucleoside or nucleotide were dissovled in D_2O or H_2O in a given molar ratio, r = [Pd]/[N], where [Pd] and [N] stand for the concentrations of the Pd complex and nucleoside (or nucleotide), respectively. The pD or pH of the solution was adjusted with NaOD and DNO_3 or NaOH and HNO_3 by using a Hitachi-Horiba M-7II pH meter. The pD values were corrected by adding 0.4 to the meter readings.[32] Unless otherwise noted, the pD (pH) of the solution was adjusted to 7.5, and the solution was allowed to stand for 3 days at 37 °C in the dark before the spectral measurement.

Raman and IR Spectra. In most of the experiments, D_2O was used as the solvent, because H_2O gives Raman and IR bands around 1640 cm^{-1}, which overlap the C(6)=O stretching band of the guanine ring. In D_2O solution, N(1) and the amino group at C(2) are deuteriated immediately after dissolution and the C(8) hydrogen atom exchanges slowly with the solvent deuterium. This exchange cannot be neglected on a time scale of days. Accordingly, for D_2O solution, we used C-(8)-predeuteriated guanine derivatives, Guo(d4), GMP(d4), and ^{18}O-Guo(d4), while nondeuteriated Guo and GMP (Guo(d0) and GMP(d0)) were used for H_2O solution.

Raman spectra were excited with 514.5- or 488.0-nm light of an Ar^+ laser (NEC GLG3302 or CR Model 52). The light scattered from the sample in a spinning cell or in a glass capillary tube was dispersed by a double monochromator (JASCO CT-80D) and detected either with a photomultiplier (Hamamatsu Photonics R464) and photon-counting electronics or with a multichannel detection system (Princeton Instruments, SMA). The concentration of G was 25–200 mM. The wavenumber axis was calibrated with indene, and peak positions were reproducible within ±1 cm^{-1} for sharp bands and ±3 cm^{-1} for broad bands.

Infrared spectra were recorded on a JASCO IR-810 infrared spectrophotometer under N_2 atmosphere. The concentration of G was 25 or 50 mM. A CaF_2 or ZnSe liquid cell was used with a sample thickness of 0.1 or 0.05 mm. The wavenumber axis was calibrated with indene. Peak positions were reproducible within ±1 cm^{-1}.

CD Spectra. Circular dichroism (CD) spectra were obtained with a JASCO J-500X spectropolarimeter. The concentration of G was 0.1–12.5 mM and the path length of the optical cell was 0.1–10 mm.

Chromatography. Gel chromatography was performed on a Sephadex G-25 Super Fine (Pharmacia Fine Chemicals) column (2.5 × 55 cm) using 0.1 M $NaNO_3$ aqueous solution as eluent at a flow rate of 10–15 mL/h. The nucleoside or nucleotide in the effluent was detected by UV absorbance at 260 nm.

High-performance liquid chromatography (HPLC) was carried out on a JASCO 880-PU liquid chromatograph with a column of Finepak SIL $C_{18}S$, Finepak GEL SA-121 (JASCO), or Asahipak GS-320 (Asahi Chemical Industry). The column was eluted at 0.5–1.0 mL/min. The eluent was H_2O/CH_3CN (95/5) containing 5 mM KH_2PO_4, 1–10 mM tetrabutylammonium bromide, and 0–10 mM sodium tartrate for the $C_{18}S$ column, 25–50 mM KH_2PO_4 + Na_2HPO_4 for SA-121, or 0.1 M potassium phosphate at pH 7.5 for GS-320. The absorbance of the effluent was monitored at 260 nm.

NMR Spectra. Nitrogen-15 NMR spectra were measured at 40.40 MHz on a JEOL JNM-GX400 spectrometer or at 50.50 MHz on a JNM-GX500 spectrometer with an external reference of 0.5 M $Na^{15}NO_3$ in D_2O. The 15 000–61 000 free induction decays were accumulated with a 30° pulse angle, and interpulse time of 2.14–2.51 s, and no proton decoupling. The concentration of GMP was 200 or 400 mM in D_2O or H_2O/D_2O (9/1).

Proton NMR spectra were measured at 500.00 MHz on the JNM-GX500 with an external reference of 1% TMS in deuteriated chloroform. A 45° pulse angle and a 4.14- or 26.64-s interpulse time were employed. The concentration of nucleoside or nucleotide was 50 mM in D_2O.

Phosphorus-31 NMR spectra were measured at 202.35 MHz on the JNM-GX500 spectrometer using 85% H_3PO_4 as an external reference. Data without proton decoupling were accumulated for 100 decays using a 90° pulse angle and a 2.40-s interpulse time. The concentration of GMP was 50 mM in D_2O.

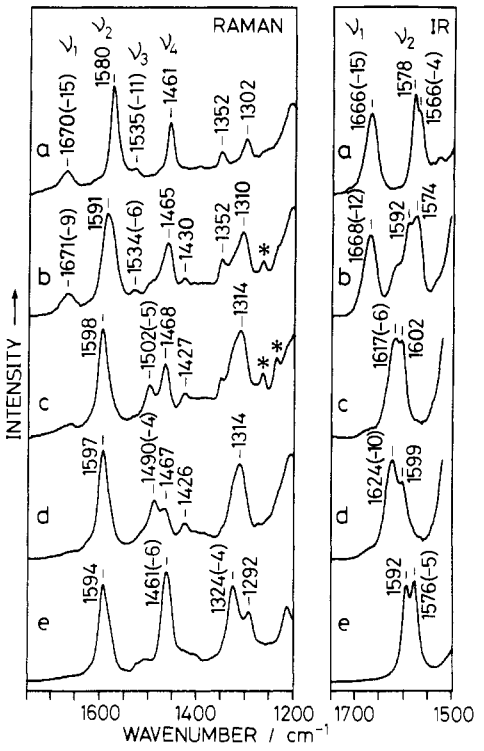

Figure 2. Raman and IR spectra of D_2O solutions of (a) Guo(d4) (pD 7.5, 80 °C), (b) Guo(d4) + $[Pd(dien)(NO_3)]NO_3$ (r = 1, pD 7.5; A-(Guo)), (c) Guo(d4) + $[Pd(dien)(NO_3)]NO_3$ (r = 2, pD 7.5; B1(Guo)), (d) Guo(d4) + $[Pd(en)(NO_3)_2]$ (r = 1, pD 7.5; B2(Guo)), and (e) Guo(d4) (pD 12; deprotonated Guo). The concentration of Guo was (a) 25 mM or (b–e) 50 mM. The bands marked with asterisks are due to Pd(dien).

Results

Raman and IR Spectra. Figure 2 shows the Raman and IR spectra of D_2O solutions of Guo(d4), Pd(dien)–Guo mixtures (r = 1, 2), and a Pd(en)–Guo mixture (r = 1) at pD 7.5 and Guo at pD 12. The observed frequency shifts on ^{18}O substitution are given in parentheses for the bands that showed shifts greater than 3 cm^{-1}. In neutral solution of Guo(d4) (Figure 2a), the IR band at 1666 cm^{-1} (1670 cm^{-1} in the Raman, to be termed ν_1) shows the largest downshift (15 cm^{-1}), and it is assigned to the C(6)=O stretching vibration. A comparable ^{18}O shift is observed for the 1535-cm^{-1} Raman band (ν_3), which is assigned to a hybrid mode of the C=O stretch and the C(5)=C(6) stretch (mode 10[33]). The

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strongest Raman band at 1580 cm^{-1} (ν_2) is insensitive to the ^{18}O substitution and assigned to a stretching mode in both pyrimidine and imidazole rings on the basis of ^{15}N shifts.[34] The 1461-cm^{-1} Raman band (ν_4) is due to an imidazole ring vibration involving the CN stretching and the C(8)H bending, as suggested by ^{15}N shifts[34] and a large C(8)D shift.[31]

In basic solution (pD 12), N(1) of Guo is deprotonated. As a result, the ν_1 and ν_3 bands disappear from the 1670- and 1535-cm^{-1} regions (Figure 2e). The IR band at 1576 cm^{-1}, the highest frequency one of the bands sensitive to the ^{18}O substitution, is assignable to ν_1 in the deprotonated state. The deprotonation makes ν_2 upshift by 14 cm^{-1} to 1594 cm^{-1} in the Raman spectrum and 1592 cm^{-1} in the IR spectrum, while the ν_4 frequency (1461 cm^{-1}) seems to remain unchanged. However, the 1461-cm^{-1} Raman band of deprotonated Guo(d4) is sensitive to the ^{18}O substitution. We assigned this band to an overlap of ν_3 and ν_4, ν_3 being downshifted by 74 cm^{-1} from 1535 cm^{-1} and ν_4 being unshifted on deprotonation.

The assignments of ν_1–ν_4 and their frequency shifts on N(1) deprotonation are useful to investigate the Pd binding sites on the guanine ring. Since ν_1 and ν_3 involve the C(6)=O stretch significantly and show large downshifts in frequency on N(1) deprotonation, the frequencies of these bands are expected to reflect the structure in the N(1)–C(6)=O part. On the other hand, ν_4 is due to a vibration primarily localized at the imidazole ring and this frequency may be affected by Pd binding to N(7), the possible binding site in the imidazole ring.

The binding of Pd(dien) affects certain Raman and IR bands, as shown in Figure 2b,c. At r = 1 (Figure 2b), ν_4 of Guo(d4) shifts to 1465 cm^{-1} and ν_2 shifts to 1591 cm^{-1}, whereas ν_1 and ν_3 show no prominent changes. At r = 2 (Figure 2c), the Raman and IR spectra are characterized by the disappearance of ν_1 and ν_3 from the 1666- and 1535-cm^{-1} regions and the appearance of an IR band at 1617 cm^{-1} and a Raman band at 1502 cm^{-1}, which are assignable to downshifted ν_1 and ν_3, respectively, on the basis of ^{18}O shifts. The ν_2 and ν_4 bands are observed at 1598 and 1468 cm^{-1}, respectively. At $1 < r < 2$, the Raman and IR spectra were sums of those at r = 1 and 2, and at $2 < r < 4$, the ν_1–ν_4 frequencies were identical with those at r = 2. These findings show that there are two types of adducts as far as the Pd binding sites in the guanine ring are concerned. One of the two adduct types is most populated at r = 1 (characterized by a set of ν_1–ν_4 frequencies (1668, 1591, 1534, 1465 cm^{-1}) and to be termed adduct A). The other type (characterized by a set of frequencies (1617, 1598, 1502, 1468 cm^{-1}) and termed adduct B) is dominant at $r \geq 2$. The spectral changes of ν_1–ν_4 associated with the adduct formation are summarized as follows. The A adduct formation causes upshifts of the ν_2 and ν_4 bands, leaving the ν_1 and ν_3 bands almost unaffected. The shift of ν_4 suggests Pd–N(7) binding. The B adduct formation leads to further upshifts of ν_2 and ν_4 and large downshifts of ν_1 and ν_3, which are suggestive of Pd binding both at N(7) and the N(1)–C(6)=O part.

In the r = 1 mixture of Pd(en) and Guo(d4) (Figure 2d), ν_1 and ν_3 downshift to 1624 and 1490 cm^{-1}, respectively. Upshifts of ν_2 and ν_4 to 1597 and 1467 cm^{-1}, respectively, are also caused by the mixing. This spectral pattern is similar to that observed for the B adduct of Pd(dien) and Guo(d4), suggesting that an adduct of type B is formed also in this case. However, small differences in the ν_1 and ν_3 frequencies compared with those of the Pd(dien) adduct (7 and 12 cm^{-1}, respectively) indicate different perturbation on the ring structure. To distinguish these two adducts, we call the one formed wth Pd(dien) B1 and the other formed with Pd(en) B2.

In the Raman and IR spectra of the B1 and B2 adducts, no effects of raising pD from 7.5 to 10 were observed. This suggests that N(1) is already deprotonated at pD 7.5 in these adducts. In highly acid solution (pD 1.5), where N(1) is hardly deprotonated,

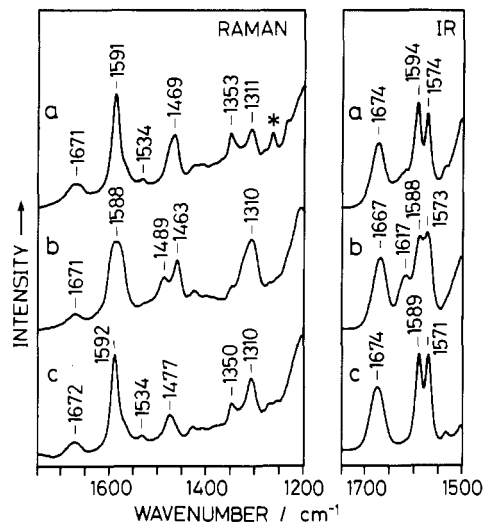

Figure 3. Raman and IR spectra of D_2O solutions of (a) Guo(d4) + [Pd(dien)(NO_3)](NO_3) (r = 2, pD 1.2), (b) Guo(d4) + [Pd(en)(NO_3)$_2$] (r = 0.5, pD 7.5, 80 °C), and (c) Guo(d4) + [Pd(en)(NO_3)$_2$] (r = 0.5, pD 4.4). The concentration of Guo was 50 mM. The band marked with an asterisk is due to Pd(dien).

the B adduct formation did not take place. Figure 3a demonstrates that only A is formed in a highly acid solution of the Pd(dien)–Guo(d4) mixture even at r = 2 in contrast to the predominant formation of B1 at neutral pD. Similar hindrance to the B2 adduct formation was also observed in the spectrum of Pd(en)–Guo(d4) solution at r = 2 and pD 1.5. All these findings indicate that N(1) is deprotonated in B1 and B2 as a result of or a prerequisite condition for the Pd binding. To confirm this, we examined pH changes upon adduct formation. When Guo powder was added to an aqueous solution of Pd(en) at pH 3.2, where the labile ligands of Pd were expected to be H_2O instead of OH^- at neutral pH,[35] the pH decreased to 1.8. The net increase in proton concentration was close to that expected for a single proton release from a guanine ring.

Another interesting effect of pD was observed for the r = 0.5 mixture of Pd(en) and Guo. The Raman or IR spectrum of this mixture at neutral pD (Figure 3b) is explained as a sum of those at r = 1 and 0 (Figure 2a,d), indicating that B2 and uncomplexed Guo(d4) coexist in the neutral pD solution. On the other hand at pD 4.4, an A-type adduct, instead of B2 at neutral pD, is formed, as shown in Figure 3c.

The adduct formation observed for Guo has been found also for GMP. The peak frequencies of the Raman and IR bands coincide with those of the Guo(d4)–Pd adducts within 10 cm^{-1}, and the intensity distributions are closely correlated wtih each other.

^{15}N NMR Spectra. The results of Raman and IR spectroscopy suggested that Pd atoms bind to N(7) and the N(1)–C(6)=O part in the B adducts. To confirm this, we measured the ^{15}N NMR spectra of B1 and B2 adducts formed with GMP (hereafter abbreviated as B1(GMP) and B2(GMP)). The peak assignments for free GMP (Figure 4a) have been made by Markowski et al.:[36] a doublet at −140.5 ppm (J = 10.4 Hz) assigned to N(7); a doublet at −207.0 ppm (J = 7.9 Hz) assigned to N(9); singlets at −210.9 and −229.0 ppm assigned to N(3) and N(1), respectively; a very weak peak at −303.0 ppm (not clear in the figure) assigned to N(2). In each of the spectra of B1(GMP) (Figure 4b) and B2(GMP) (Figure 4c), no signal is detectable around −140 ppm but two doublet signals are observed at −203 and −220 ppm, one of which must be assigned to N(7) and the other to N(9). The large upfield shift of the N(7) signal upon the adduct formation suggests that N(7) binds to Pd in the B adducts. The signals around −298 ppm in Figure 4b,c are certainly assigned to N(2)

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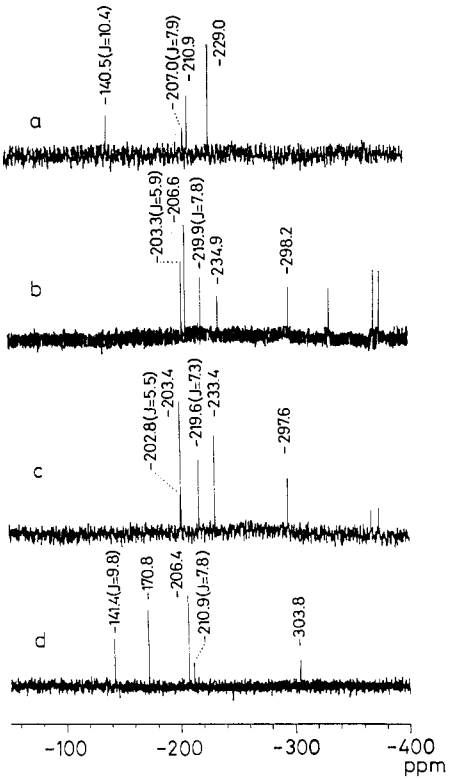

Figure 4. Nitrogen-15 NMR spectra of (a) GMP, (b) B1(GMP), (c) B2(GMP), and (d) deprotonated GMP in D_2O solution. The pD value was (a–c) 7.5 or (d) 12.0. The concentration of GMP was (a, c) 200 mM or (b, d) 400 mM. Samples of B1(GMP) and B2(GMP) were obtained by incubation at 37 °C for 3 days. Coupling constants in hertz are given in parentheses.

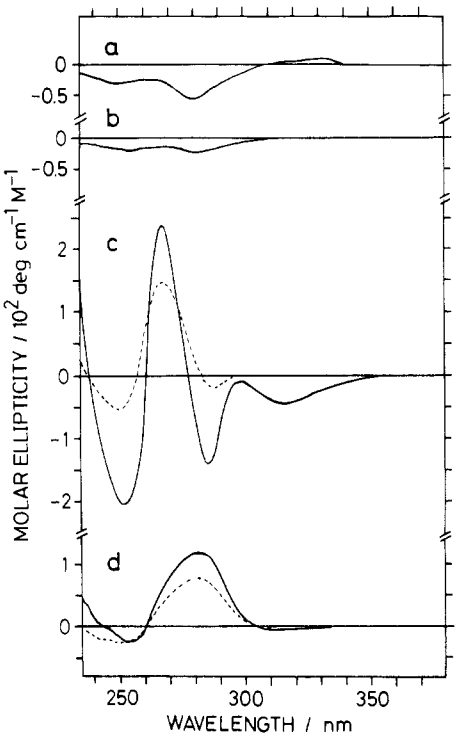

Figure 5. Circular dichroism spectra of (a) B1(GMP), (b) B1(Guo), (c) B2(GMP), and (d) B2(Guo) at pH 7.5. The concentration of GMP or Guo was 12.5 mM, and the path length was 0.10 mm. The solid spectra were obtained for the samples kept at 37 °C for 3 days after preparation, and the dashed spectra were measured soon after sample preparation.

because they become triplets in H_2O/D_2O (9/1) solution. The chemical shift of N(2) is close to that in free GMP, indicating that N(2) is not the Pd binding site. In deprotonated GMP (Figure 4d), the peak at −170.8 ppm is assigned to N(1).[37] However, no signal around −170 ppm is observed for the B adducts (Figure 4b,c) but a singlet due to N(1) seems to remain in the region between −200 and −235 ppm. This is understood if the large downfield shift due to the N(1) deprotonation is counteracted by a large upfield shift upon Pd coordination to the N(1)–C(6)=O part.

Chromatography. Gel chromatography was performed to estimate the molecular sizes of the B adducts. The B2 adduct eluted faster than the B1 adduct, indicating that the molecular size of the former is larger than that of the latter. This finding is consistent with that the latter structure is $\{Pd(dien)\}_2(G)$ and the B2 adduct consists of n molecules each of Pd(en) and G. A small difference in the elution volume between B2(Guo) and B2(GMP) may be ascribed to the presence or absence of the phosphate group. The HPLC chromatograms of B2(GMP) and B2(Guo) gave a single sharp peak under several elution conditions. Accordingly, neither of them is a mixture of oligomers of $\{Pd(en)\}_n(G)_n$ of different n values, but an oligomer of a certain n value. It is pointed out here that such an oligomer is formed immediately after mixing of Pd(en) and G.

A mixture of Pd(en) and GMP at $r = 0.5$ and at neutral pH was separated into B2(GMP) and free GMP by gel chromatography, which is consistent with results found by vibrational spectrscopy. It is interesting to note that 1/2 complex $Pd(en)(G)_2$ is not formed at neutral pH.

CD Spectra. Circular dichroism spectra of the B1 and B2 adducts are shown in Figure 5. In contrast to the spectra of B1(GMP) and B1(Guo) showing weak CD bands (Figure 5a,b), the spectrum of B2(GMP) (solid line of Figure 5c) shows strong bands at 284, 268, and 252 nm and a weak, broad band around 314 nm. In the spectrum of B2(Guo) (Figure 5d), a mediumly strong positive band and a weak negative band are observed. These observations demonstrate that the interactions among G rings in B2 are more stereospecific than in B1 and they are enhanced by the phosphate group.

CD spectra of B2(GMP) were independent of concentration in a range 0.1–12.5 mM GMP. The CD intensities decreased only about 25% even when the temperature was raised to 75 °C from the room temperature. These findings also indicate a stabilized configuration of the G molecules in B2(GMP). The intensities of the CD bands of B2(GMP) increased gradually with time during 3 days after preparation of the sample (Figure 5c). Such a time-dependent change was not noticed for B1(GMP), B1(Guo), and B2(Guo).

^{1}H and ^{31}P NMR Spectra. The adduct of B2(GMP) incubated for 3 days at 37 °C gave simple ^{1}H and ^{31}P NMR spectra. In the ^{1}H NMR spectrum, signals due to C(8)–H and C(1′)–H[24] were observed at 8.27 and 6.50 ppm, respectively. Other signals at 4.53, 4.47, 4.39, 4.27, and 4.16 ppm were assigned respectively to C(3′)–H, C(4′)–H, C(5′)–H, C(2′)–H, and C(5′)–H on the basis of spin–spin-decoupling measurement. Phosphorus-31 NMR spectrum showed a single sharp signal at 5.1 ppm with a downfield shift from 4.6 ppm of free GMP. The ^{1}H and ^{31}P NMR spectra indicate that a single GMP species exists in B2(GMP). On the other hand, the ^{1}H and ^{31}P spectra of B2(GMP) immediately after sample preparation gave several signals in each signal region and were much more complicated than those of the same sample after 3 days. B2(Guo) did not give sharp ^{1}H signals even several days after sample preparation. These observations suggest that the environments of GMP in B2(GMP) are not uniform right after the mixing and they become uniform as the several guanosine conformers are converted into the most stable one. Possibly, the phosphate groups contribute to the stabilization. B1(GMP) and B1(Guo) consist of one-adduct species even immediately after mixing, judging from the simple and unchanged spectral patterns.

B2-Type Adducts Formed with Mixtures of GMP and IMP. Häring and Martin have proposed that IMP interacts with Pd(en) in a way similar to that of GMP and Pd(en) on the basis of ^{1}H NMR spectra.[24] Actually, when B2(IMP) was chromatographed

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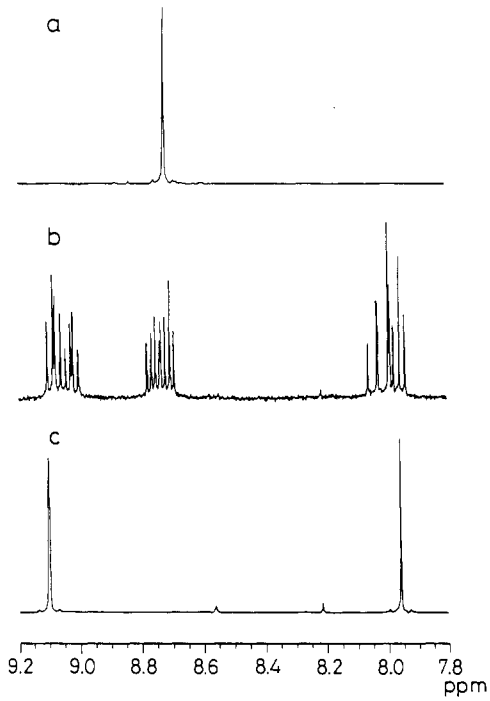

Figure 6. Proton NMR spectra of (a) B2(GMP), (b) Pd(en)–GMP/IMP(1/1), and (c) B2(IMP) in the region of purine-ring protons. All the spectra were measured 3 days after sample preparation. IMP proton signals are assigned as follows from downfield. C(8)–H: GI_3, *trans*-G_2I_2, I_4, GI_3, *cis*-G_2I_2, G_3I, GI_3, and *cis*-G_2I_2. C(2)–H: *cis*-G_2I_2, GI_3, G_3I, *trans*-G_2I_2, GI_3, *cis*-G_2I_2, I_4, and GI_3.

over Sephadex G-25, the adduct eluted as fast as B2(GMP) at neutral pH, demonstrating that the IMP adduct was also formed from the same n molecules each of Pd(en) and IMP as in the case of B2(GMP). Several ^{31}P NMR signals of IMP were observed around 3.9–4.8 ppm soon after mixing and were converted into one peak at 4.8 ppm after 3 days. The magnitude of a negative peak at 272 nm in the CD spectrum increased gradually with time and reached a constant value after several days. These observations support the proposal by Häring and Martin.[24] Here, ^1H NMR spectra of B2-type adducts of Pd(en) and mixtures of GMP and IMP in various molar ratios ([G]/[I]) (abbreviated as Pd(en)–GMP/IMP([G]/[I])) were studied.

Figure 6 shows the ^1H NMR spectra of B2(GMP), Pd(en)–GMP/IMP(1/1), and B2(IMP) in the purine proton region 3 days after sample preparation. B2(IMP), as well as B2(GMP), gives a simple spectrum as reported.[24] Pd(en)–GMP/IMP(1/1), on the other hand, gives eight signals each in the regions of C(8)–H of GMP and C(8)–H and C(2)–H of IMP. When the ratio [G]/[I] is changed, the peak positions remain unchanged but the intensity pattern changes. Thus, the complex spectra of the B2-type adducts must arise from $[\{Pd(en)\}_n(GMP)_m(IMP)_{n-m}]$, where $m = 0$–n. The appearance of eight signals is in sharp contrast to the observation that only one signal appears in each region for the B1-type adducts formed from the mixtures of GMP and IMP (or Guo and Ino).

Discussion

The vibrational spectra reported above have shown that three types of adducts can be formed between the Pd complexes (Pd(dien) and Pd(en)) and guanine derivatives (GMP and Guo). The type of adduct to be formed depends on the possible number of ligation sites in each of the Pd complexes and guanine derivatives at a given pD and molar ratio r of the former to the latter. The spectral dependence on pD is related to the protonation and deprotonation at N(1), which is one of the possible Pd binding sites of a guanine ring.

In the neutral 1/1 mixture of Pd(dien) and Guo, adduct A is formed mainly. Since each molecule of Pd(dien) has one ligation site, it coordinates to a guanine ring at one site. According to an X-ray diffraction study,[38] the Pd ion binds to N(7) of Guo in a crystal of $[Pd(dien)(Guo)](ClO_4)_2$. Figure 7 compares the Raman spectrum of this compound and that of uncomplexed Guo in the solid state. On N(7) binding, the ν_2 (1577 cm^{-1}) and ν_4 (1480 cm^{-1}) bands shift to 1598 and 1503 cm^{-1}, respectively, while the ν_1 band remains around 1700 cm^{-1}. The two upshifted bands correspond to the 1580 (ν_2) and 1461 cm^{-1} (ν_4) bands of Guo(d4) in D_2O solution (Figure 2b), which have also upshifted on formation of adduct A. It has been reported that ν_4 shifts up by coordination of other metals (Pt, Ni) at N(7).[4,6] The relative insensitiveness of ν_1 to the N(7) binding parallels the similar observation with the A adduct formation. Thus, Pd(dien) is concluded to bind to N(7) in adduct A. The structure may be written as $Guo^{N(7)}$–Pd(dien). In acid solution, Pd(en) also has been found to form adduct A. Such an adduct formed with Guo at $r = 0.5$ and pD 4.4 is considered to be a 1/2 complex, $Guo^{N(7)}$–Pd(en)–$Guo^{N(7)}$.

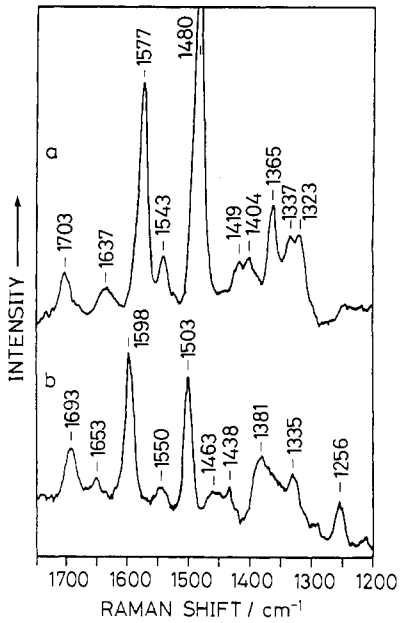

Figure 7. Raman spectra of (a) Guo(d0) and (b) [Pd(dien)(Guo(d0))]$(ClO_4)_2$ in the solid state.

The B-type adducts have been found to form predominantly in neutral solutions of GMP or Guo mixed with Pd(dien) at $r = 2$ (B1) and with Pd(en) at $r = 1$ (B2). Resemblance of Raman and IR spectra between the two adducts including the ^{18}O shifts suggests that the same two sites of a guanine ring are used in the formation of B1 and B2. One of the sites must be N(7), because ν_4 upshifted as in the case of adduct A formation. As the second site, deprotonated N(1) or O(6) is suggested by the frequency shifts of ν_1 and ν_3.

The C(6)^{18}O substitution of Guo has enabled us to find two series of Raman and/or IR bands contributed from the ν_{CO} vibration, ν_1 and ν_3. From the frequencies of these bands it has been shown that the electronic structures in the N(1)–C(6)=O parts in B1 and B2 are intermediate between those of N(1)-protonated and -deprotonated states of the unligated guanine derivatives. In the deprotonated state, the negative charge is supposed to delocalize from N(1) and to distribute over the ring, in particular the C(6)O vicinity.[39] The behavior of ν_1 and ν_3 upon the B adduct formation, however, is accounted for by assuming that the delocalized negative charge is partly taken back to N(1) in the B adducts. Such movement of charge is not simply expected for the binding at any of C(2)N, N(3), and C(6)O. On the other hand, if the Pd ion binds to N(1), the positive charge of the ion may attract the negative charge and induce the expected effect on these vibrations. Accordingly, we conclude that the second Pd binding site is N(1). The ^{15}N NMR spectra of B adducts are

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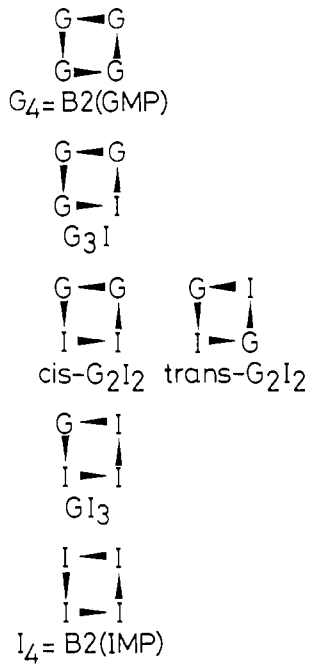

Figure 8. Six possible G_mI_{4-m} ($0 \le m \le 4$) adducts. Arrows are used to represent Pd(en) molecules and to distinguish between the N(7) and N(1) bindings.

also interpreted in a similar manner and give support to the conclusion.

On the basis of the observations described in the previous section, adduct B1 is Pd(dien)–$G^{N(7),N(1)}$–Pd(dien), while B2 is considered to take a form of $[\{Pd(en)\}_n(G^{N(7),N(1)})_n]$, where n is a definite number. For the B2 adduct formed with GMP (B2(GMP)), various guanosine conformers are converted into the most stable one during a few days. Once the most stable conformer is formed, the structure does not change much by raising the concentration or temperature. We discuss the value n and the structure of B2 in the following paragraphs.

Binding sites and n in B2(IMP) are considered to be the same as those in B2(GMP) on the basis of similar physicochemical characteristics described in the preceding section. Hence, adducts of $[\{Pd(en)\}_n(GMP)_m(IMP)_{n-m}]$ (abbreviated as G_mI_{n-m}), where $0 \le m \le n$, must be formed in the Pd(en)–GMP–IMP mixtures. In the ^{1}H NMR spectra of B2(GMP) and B2(IMP) only one signal in each proton region was observed, while eight each were observed with various intensity patterns for the B2 adducts formed with GMP/IMP mixtures. The former observation signifies that all the C(8)–H protons of GMP (or all the C(8)–H and C(2)–H protons of IMP) are located in symmetrically equivalent positions, which suggests that the n/n adduct takes a cyclic structure of C_n symmetry (at least for the purine bases). The latter observation indicates that such a high symmetry does not hold in the mixed adducts, and eight protons in slightly different environments are present in the mixtures. This has turned out to be best explained by a cyclic head-to-tail structure of $n = 4$ as described below.

In the case of $n = 4$ the mixed adducts consist of G_4, G_3I, *cis*-G_2I_2, *trans*-G_2I_2, GI_3, and I_4 (Figure 8). Then the eight signals in the C(8)–H region of GMP arise from the following five species: one from G_4, three from G_3I, two from *cis*-G_2I_2, one from *trans*-G_2I_2, and one from GI_3. The origin of each signal is assigned as follows on the basis of the intensity patterns for various [G]/[I] ratios (Table I, the eight signals of C(8)–H of GMP are termed a–h from the downfield side). Signal f must arise from G_4 because the relative intensity becomes largest for high [G]/[I] ratios. Signal c is assigned to GI_3 because it is strongest for the 1/3.4 mixture. The three G signals of G_3I or the two G signals of *cis*-G_2I_2 must have similar intensity characteristics. Thus, signals b, e, and h are assigned to G_3I, and a and d, to *cis*-G_2I_2. The remaining signal, g, is assigned to *trans*-G_2I_2. Assignments for the C(8)–H and C(2)–H signals of IMP are made in a similar manner, and they are given in the caption to Figure 6.

Table I. ^{1}H NMR Signal Intensity Distribution (%) in the C(8)–H Region for Mixtures of Pd(en) and GMP/IMP with Varied [G]/[I] Ratios[a]

[G]/[I]	a	b	c	d	e	f	g	h
3/1	4	12	3	18[b]		42	9	13
	4.7	14.1	1.6	4.7	14.1	42.2	4.7	14.1
2/1	7	14	7	9	12	28	13	12
	7.4	14.8	3.7	7.4	14.8	29.6	7.4	14.8
1/1	10	12	16	8	10	13	20	12
	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1/3.4	10	6	40	9	5	4	21	4
	13.6	4.0	46.1	13.6	4.0	1.2	13.6	4.0
assgnt	*cis*-G_2I_2	G_3I	GI_3	*cis*-G_2I_2	G_3I	G_4	*trans*-G_2I_2	G_3I

[a] Signals are termed alphabetically from the downfield side. The observed intensities (upper) were compared with the calculated intensities (lower) in the binomial distribution approximation. [b] Signals d and e were unresolved for this mixing ratio.

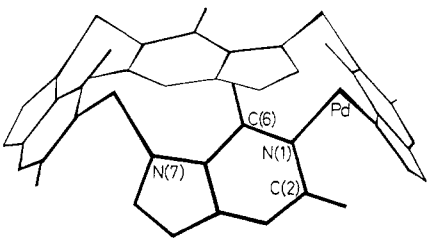

Figure 9. Adduct structure proposed for B2(GMP). Only the guanine rings and Pd atoms are shown.

In Table I are compared the observed and calculated intensities by assuming the binomial distribution for the populations of the five G-containing-adduct species. The observed intensity pattern is generally well reproduced in the calculations for the four mixing ratios. A systematic difference between the observed and calculated is seen for the *trans*-G_2I_2 signals, indicating that the *trans*-G_2I_2 form is particularly stable.

The validity of the head-to-tail linkage was confirmed by the following observation for a mixed-adduct GI_3. When signal c (C(8)–H of G in GI_3) was irradiated, homonuclear Overhauser effects were observed only for one signal in the C(2)–H region of I (the fifth signal from downfield), indicating that the C(8)–H of G in the GI_3 adduct is located in the vicinity of a C(2)–H but not of a C(8)–H of I. Figure 9 shows the structure consistent with the observations, where only guanine rings and Pd atoms are depicted.

As mentioned above, various conformers existing just after mixing are converted into one stable rigid structure of B2(GMP) within a few days. On the other hand, such conversion does not take place for the Pd(en)–Guo mixture, B2(Guo), as suggested from its ^{1}H NMR and CD spectra. B2(Guo) is also considered to be formed from four molecules each of the components, since there is no significant difference in elution rate of gel chromatography between B2(GMP) and B2(Guo). The formation of a rigid structure of B2(GMP) as compared to various conformers in B2(Guo) seems to be ascribable to the presence of a phosphate group in the former. The four phosphates are in the same environment in B2(GMP), which is different from that of free GMP, judging from the ^{31}P NMR signals. In order to examine the role of the NH_2 groups of Pd(en) in the stabilization of B2(GMP), Pd(tmen) was used instead of Pd(en). While the IR (ν_1 at 1623 cm^{-1}) and Raman (ν_3 at 1494 cm^{-1}) spectra of the Pd(tmen)–GMP adduct suggested the same coordination sites as those in B2(GMP), many signals were observed in the C(8)–H ^{1}H NMR spectra and the CD spectral pattern was different from that of B2(GMP). These observations are analogous to those for B2(Guo). Hydrogen bonding between PO_3^{2-} in GMP and NH_2 in Pd(en) must contribute to stabilization of a specific confromer by tightening the purine and ribose rings at a certain configuration in B2(GMP). Actually a small downshift ($\le$2 cm^{-1}) of the phosphate Raman band at 978 cm^{-1} on B2(GMP) formation was observed, which indicates the hydrogen-bond formation. Direct Pd interactions with phosphate are certainly absent because such interactions

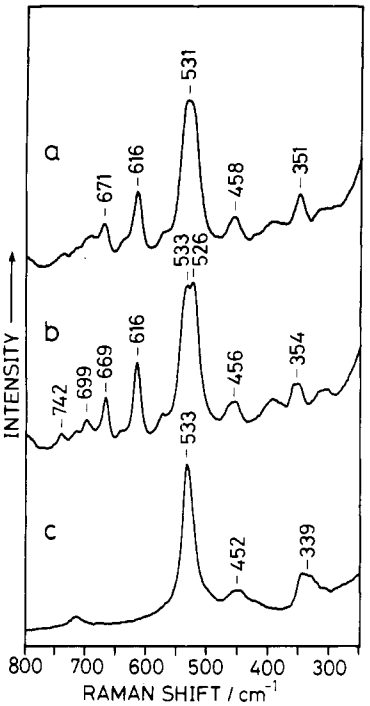

Figure 10. Raman spectra of H_2O solutions (pH 7.5) of (a) GMP(d0) + $[Pd(en)(NO_3)_2]$ (r = 1, 200 mM) recorded soon after sample preparation, (b) the same sample as (a) but recorded after incubation at 37 °C for 3 days, and (c) $[Pd(en)(NO_3)_2]$ (500 mM).

would upshift the band by several wavenumbers.[40]

The structural information about the Pd(en) part is obtained from Raman spectra. The Raman spectra of B2(GMP(d0)) recorded soon after sample preparation and after incubation for 3 days are compared with the spectrum of free Pd(en) in Figure 10. A strong band around 530 cm^{-1} is assigned to the Pd–NH_2 symmetric stretching mode.[41] This band of the B2 adduct is broader than that of free Pd(en) (fwhm = 24 cm^{-1}), even immediately after sample preparation (33 cm^{-1}), and splits into two peaks after 3 days (38 cm^{-1}). These spectral changes are explained as follows. Pd(en) can take two forms whose NC–CN torsional angle is gauche or gauche′. Since the normal frequencies of the two conformers are the same, a single sharp peak is observed in the free Pd(en). When Pd is bound unsymmetrically to guanine N(1) and N(7), it is possible for the two conformers to give slightly different normal frequencies, which may be a reason of the band broadening immediately after mixing. Meanwhile, the PO_3^{2-} group of GMP can be hydrogen bonded from one NH in one of the two NH_2 groups. Possibly, hydrogen bonding with one conformer of Pd(en) may be easier to form and stronger than that with the other. Such interactions with the phosphate group will further split the frequencies of the two conformers, since the 530-cm^{-1} vibration involves NH_2 motion as judged from a significant shift (−40 cm^{-1}) on deuteriation. Of the two peaks of B2(GMP), one at 526 cm^{-1} is tentatively assigned to the conformer with a strong H-bond, and the other at 533 cm^{-1}, to that with a weak H-bond. Accordingly, the strict C_4 symmetry does not hold for the en and phosphate parts on the molecular vibrational time scale. Such a fast equilibrium between two conformers with different hydrogen bonds may not be separately observed on the NMR time scale and, actually, a singlet peak was observed in the ^{31}P NMR spectrum.

The Raman bands due to GMP in the 600–700-cm^{-1} region are known to be sensitive to the ribose ring pucker and N(9)–C(1′) conformation.[42,43] These bands become sharper after incubation in accordance with the simplification of CD and NMR spectra, which is also explained by enrichment of a certain conformer as a result of the hydrogen bonding between en and phosphate parts.

It is interesting to compare the binding modes of Pd to the guanine ring at neutral pH with those of Pt. Here we have found that Pd binds to N(7) and deprotonated N(1) to form 1/1 $G^{N(7)}$–Pd (A) and 2/1 Pd–$G^{N(7),N(1)}$–Pd (B1) adducts and a 4/4 cyclic adduct (B2) with N(7)–Pd–N(1) cross-linking. A 1/2 adduct, $G^{N(7)}$–Pd–$G^{N(7)}$, has also been found at acid conditions, but such an adduct has not been detected at neutral pH. In contrast to these Pd binding modes, most of the studies on the Pt binding modes reported so far suggested the N(7)–Pt binding and the N(7)–Pt–N(7) cross-linking as the major binding modes.[4-9] Such cross-linking of adjacent guanine bases in DNA by *cis*-DDP is thought to be the key step in antitumor activity of this platinum drug. The lack of N(1) binding in the Pt adducts indicates that Pt is less reactive than Pd and can bind only to N(7), the most reactive site of the guanine ring. On the other hand, Pd is so reactive that it can replace the N(1) proton when the N(7) is already bound to another Pd atom. Such high reactivity of Pd complexes may not be directed only to nucleic acids but also to proteins. The binding to proteins may partly account for the finding that some Pd complexes inhibit DNA synthesis *in vitro* similarly to *cis*-DDP, but such activity of the Pd complexes is greatly reduced in the presence of proteins.[44] *trans*-DDP also has higher reactivity with proteins than *cis*-DDP and is inactive as an antitumor drug.[45]

Conclusion

Pd(dien), which has one ligation site, forms two types of adducts with Guo or GMP at neutral pH. In the 1/1 adduct Pd binds to N(7), whereas two Pd atoms bind to N(7) and deprotonated N(1) of one guanine ring in the 2/1 adduct. On the other hand, Pd(en) having two ligation sites in the cis configuration predominantly forms a 4/4 cyclic adduct with N(7)–Pd-deprotonated N(1) linkages between adjacent guanine rings. The cyclic adduct formed with GMP molecules is stabilized by hydrogen bonding from the amino groups of en to the phosphate groups. No evidence has been found for the 1/2 adduct formation with an N(7)–Pd–N(7) cross-link at neutral solution, which is in sharp contrast to the predominant formation of this type of adduct by platinum complexes having two ligation sites in the cis configuration such as *cis*-DDP.

Acknowledgment. This study was supported by Grants-in-Aid for Scientific Research 57470010 and 60780293 from the Ministry of Education, Science, and Culture, Japan.

Registry No. A(Guo), 73601-42-0; B1(GMP), 120229-23-4; B1(Guo), 120205-36-9; B2(IMP), 120229-19-8; B2(GMP), 120229-20-1; GMP, 85-32-5; Guo, 118-00-3; $[Pd(dien)(NO_3)]NO_3$, 120229-25-6; $[Pd(en)(NO_3)_2]$, 63994-76-3; $[Pd(en)(Guo)_2]^{2+}$, 120205-35-8; $[\{Pd(en)\}_4(GMP)(IMP)_3]$, 120229-21-2; *trans*-$[\{Pd(en)\}_4(GMP)_2(IMP)_2]$, 120229-18-7; *cis*-$[\{Pd(en)\}_4(GMP)_2(IMP)_2]$, 120331-84-2; $[\{Pd(en)\}_4(GMP)_3(IMP)]$, 120229-22-3; D_2, 7782-39-0.

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